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(71) Applicant (for all designated States except US): FLUX-  
OME SCIENCES A/S [DK/DK]; Danmarks Tekniske  
Universitet, Bygning 223, Søtofts Plads, DK-2800 Lyngby  
(DK).

(72) Inventors; and

(75) Inventors/Applicants (for US only): BRO, Christoffer  
[DK/DK]; Snogegårdsvej 2 st. th., DK-2820 Gentofte  
(DK). REGENBERG, Brigitte [DK/DK]; Gammel  
Kalkbraenderivej 8, 1. th, DK-2100 København (DK).

NIELSEN, Jens [DK/DK]; Hans Bruuns Vej 8, DK-2920  
Charlottenlund (DK).

(74) Agent: SMART, Peter, J.; W.H. Beck, Greener & Co., 7  
Stone Buildings, Lincoln's Inn, London WC2A 3SZ (GB).

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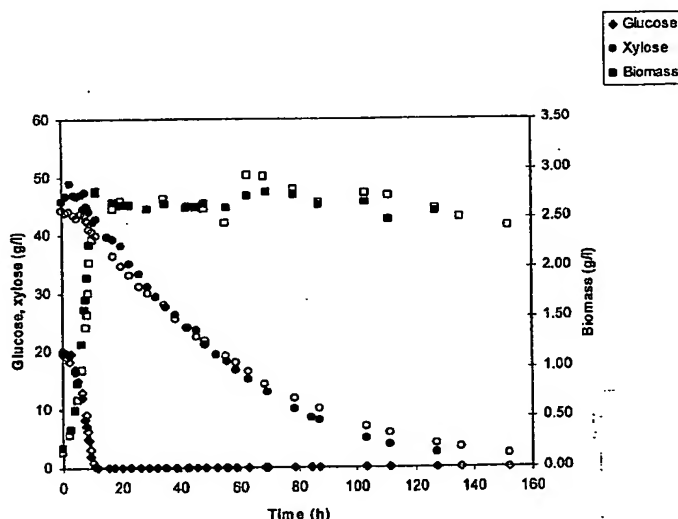
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(54) Title: METABOLICALLY ENGINEERED MICRO-ORGANISMS HAVING REDUCED PRODUCTION OF UNDESIRE  
METABOLIC PRODUCTS



(57) Abstract: A metabolically engineered micro-organism has an operative first metabolic pathway in which a first metabolite is transformed into a second metabolite in a reaction in which NAD is a cofactor for a first enzyme, suitably a phosphorylating dehydrogenase, said reaction step producing NADH. Said second metabolite is transformed into at least one further metabolite in a reaction catalysed by a second enzyme, suitably a kinase. The organism has an operative second metabolic pathway characterised by an enzyme activity in excess of a native level in respect of a third enzyme, suitably a non-phosphorylating dehydrogenase, e.g. GAPN, catalysing a non-reversible reaction in which NADP is a cofactor and NADPH is a product. Said first metabolite is transformed into a said further metabolite without the involvement of said second enzyme.

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# INTERNATIONAL SEARCH REPORT

International Application No

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## A. CLASSIFICATION OF SUBJECT MATTER

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## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, MEDLINE, WPI Data, PAJ

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>VALVERDE F ET AL: "Engineering a central metabolic pathway: glycolysis with no net phosphorylation in an Escherichia coli gap mutant complemented with a plant GapN gene"</p> <p>FEBS LETTERS, ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM, NL, vol. 449, no. 2-3, 23 April 1999 (1999-04-23), pages 153-158, XP004259550</p> <p>ISSN: 0014-5793</p> <p>cited in the application</p> <p>page 155, paragraph 1</p> <p style="text-align: center;">---</p> <p style="text-align: center;">-/--</p>	<p>1-9, 13-16</p>

☒ Further documents are listed in the continuation of box C.

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Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel (+31-70) 340-2040, Tx. 31 651 epo nl,  
Fax: (+31-70) 340-3016

Authorized officer

Aslund, J

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## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>IDDAR ABDELGHANI ET AL: "Expression, purification, and characterization of recombinant nonphosphorylating NADP-dependent glyceraldehyde-3-phosphate dehydrogenase from Clostridium acetobutylicum"</p> <p>PROTEIN EXPRESSION AND PURIFICATION, vol. 25, no. 3, August 2002 (2002-08), pages 519-526, XP002273747</p> <p>ISSN: 1046-5928</p> <p>the whole document</p>	1-9, 13-16
A	<p>BIANCHI MICHELE M ET AL: "Efficient homolactic fermentation by Kluyveromyces lactis strains defective in pyruvate utilization and transformed with the heterologous LDH gene."</p> <p>APPLIED AND ENVIRONMENTAL MICROBIOLOGY, vol. 67, no. 12, December 2001 (2001-12), pages 5621-5625, XP002236026</p> <p>ISSN: 0099-2240</p> <p>the whole document</p>	15
A	<p>NIELSEN J: "Metabolic engineering."</p> <p>APPLIED MICROBIOLOGY AND BIOTECHNOLOGY, vol. 55, no. 3, April 2001 (2001-04), pages 263-283, XP002236027</p> <p>ISSN: 0175-7598</p> <p>cited in the application</p> <p>the whole document</p>	1-16
A	<p>WANG Z ET AL: "Glycerol production by microbial fermentation - A review"</p> <p>BIOTECHNOLOGY ADVANCES, ELSEVIER PUBLISHING, BARKING, GB, vol. 19, no. 3, June 2001 (2001-06), pages 201-223, XP004255780</p> <p>ISSN: 0734-9750</p>	
A	<p>MICHNICK SUMIO ET AL: "Modulation of glycerol and ethanol yields during alcoholic fermentation in Saccharomyces cerevisiae strains overexpressed or disrupted for GPD1 encoding glycerol 3-phosphate dehydrogenase."</p> <p>YEAST, vol. 13, no. 9, 1997, pages 783-793, XP008015354</p> <p>ISSN: 0749-503X</p>	
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A	<p>VALADI H ET AL: "Improved ethanol production by glycerol-3-phosphate dehydrogenase mutants of <i>Saccharomyces cerevisiae</i>."</p> <p>APPLIED MICROBIOLOGY AND BIOTECHNOLOGY, vol. 50, no. 4, October 1998 (1998-10), pages 434-439, XP002236029</p> <p>ISSN: 0175-7598</p> <p>cited in the application</p> <p style="text-align: center;">---</p>	
A	<p>ALEXANDRE H ET AL: "Global gene expression during short-term ethanol stress in <i>Saccharomyces cerevisiae</i>"</p> <p>FEBS LETTERS, ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM, NL,</p> <p>vol. 498, no. 1, 1 June 2001 (2001-06-01), pages 98-103, XP004243333</p> <p>ISSN: 0014-5793</p> <p style="text-align: center;">---</p>	
A	<p>RODRGUEZ-ACOSTA F ET AL: "Non-linear optimization of biotechnological processes by stochastic algorithms: Application to the maximization of the production rate of ethanol, glycerol and carbohydrates by <i>Saccharomyces cerevisiae</i>"</p> <p>JOURNAL OF BIOTECHNOLOGY, ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM, NL,</p> <p>vol. 68, no. 1,</p> <p>5 February 1999 (1999-02-05), pages 15-28, XP004157315</p> <p>ISSN: 0168-1656</p> <p style="text-align: center;">---</p>	
A	<p>NISSEN T L ET AL: "Optimization of ethanol production in <i>Saccharomyces cerevisiae</i> by metabolic engineering of the ammonium assimilation."</p> <p>METABOLIC ENGINEERING. UNITED STATES JAN 2000,</p> <p>vol. 2, no. 1, January 2000 (2000-01), pages 69-77, XP002236030</p> <p>ISSN: 1096-7176</p> <p style="text-align: center;">-----</p>	